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Fungitoxic activity of four thio-functionalised glucosinolate enzyme-derived products on ten soil-borne pathogens

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Abstract: Enzyme-derived products obtained from thio-functionalised glucosinolates showed high fungitoxicity, a wide activity spectrum and special physicochemical properties, which suggest their potential as alternatives to commercial fumigants for controlling several soil-borne pathogens.

Keywords: fumigants; glucosinolates; isothiocyanates; soil-borne fungal pathogens

1 INTRODUCTION

Previous studies have reported that glucosinolate-derived products (GL-DPs) were bioactive,[1] able to control, *in vitro*, nematodes,[2] fruit post-harvest pathogens,[3,4] tumour cell proliferation,[5,6] as well as phytopathogenetic fungi.[7] Moreover, GL-DPs obtained from benzyl- and thio-functionalised glucosinolates (ThioGLs) showed a fungitoxic activity significantly higher than that of alkenyl and hydroxyalkenyl GL-DPs.[7] As the use of soil fumigants is likely to be severely limited in the near future,[8] the aim of our studies was to find natural fungitoxic compounds as possible alternatives. This paper reports the fungitoxic activity of four ThioGL-derived products (ThioGL-DPs), prepared using pure ThioGLs, towards 10 of the most economically important soil-borne pathogens.

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2 EXPERIMENTAL

2.1 In-vitro assay. The ThioGLs used in this study were glucoiberin (GIB), glucocheirolin (GCH), glucoerucin (GER) and glucoraphenin (GRE). They were isolated from *Brassicaceae* seeds[7] and purified according to Thies[9] with some modifications.[10] The ThioGL-DPs were prepared by dissolving each glucosinolate (GL) in phosphate buffer (50 mM; pH 6.5) contained in a vial, and adding a suitable amount of pure myrosinase, isolated according to Palmieri *et al*.[11] Reaction mixtures were incubated at 37°C overnight. Under these conditions, the myrosinase-catalysed hydrolysis produces essentially the corresponding isothiocyanates, as confirmed by GLC-MS. The in-vitro antifungal activity was tested towards 10 soil-borne pathogens (Tables 1 and 2) by the Poison Food Technique,[12] following the experimental design of a previous GL-DP screening.[7] The doses, chosen on the basis of preliminary trials, varied from 6 μM for *Phytophthora* and *Pythium* spp to 3 mM for Deuteromycetes and Sclerotinia.

Table 1. Effectiveness Index of myrosinase-catalysed derived products of four thiofunctionalised glucosinolates (ThioGl-DPs) *in vitro* on 10 soil-borne pathogens

	EI[a] (%)
Fungal species	
Phytophthora nicotiana	100 a
Phytophthora cactorum	90.5 b
Pythium ultimum	81.4 c
Pythium irregulare	75.7 d
Sclerotium rolfsii	72.2 d
Rhizoctonia solani	59.5 e
Pyrenochaeta lycopersici	33.3 f
Verticillium dahliae	25.7 g
Fusarium oxysporum f sp *lycopersici* r 1	17.0 h
Sclerotinia sclerotiorum	13.6 h
ThioGL-DPs from	
Glucoiberin	69.9 a
Glucocheirolin	67.5 a
Glucoerucin	47.2 b
Glucoraphenin	42.9 c
Interaction fungus – product	**[b]

[a] EI = 100 (control diameter – treatment diameter)/(control diameter); compounds tested at 0.2 mM. Means followed by a common letter are not significantly different, according to LSD test ($P \leqslant 0.05$)
[b] Significant at $P \leqslant 0.01$

Fungal species[a]	ThioGL-DPs from Glucoiberin Glucocheirolin		ThioGL-DPs from Glucoerucin Glucoraphenin	
	IC_{50} (mM)[b]	MIC (mM)[c]	IC_{50} (mM)[b]	MIC (mM)[c]
Fusarium oxysporum fsp *lycop* r1 *Verticillium dahliae* *Sclerotinia sclerotiorum* *Pyrenochaeta lycopersici*	0.2–0.6	0.4–1.2	0.5–1.5	>3
Rhizoctonia solani *Sclerotium rolfsii*	0.08–0.3	0.3–1	0.1–0.4	0.8–3
Pythium irregulare *Pythium ultimum* *Phytophthora cactorum* *Phytophthora nicotiana*	0.007–0.05	0.024–0.15	0.05–0.17	0.1–0.4

[a] Cultures were obtained from Centraalbureau Voor Schimmelcultures (*P ultimum*, 296.37; *P nicotiana*, 301.29; *V dahliae*, 390.49) and from American Type Culture Collection (*F oxysporum* fsp *lycopersici* r1, 16417), while *S sclerotiorum*, *R solani*, *S rolfsii*, *B cinerea*, *P lycopersici*, *P cactorum*, *P irregulare* (included in International Mycological Institute collection no 368281) had been previously isolated in our laboratory.
[b] Concentration (mM) required to cause a 50% inhibition of growth of fungi *in vivo*.
[c] Minimum inhibitory concentration, *in vitro*.

Table 2. Effect of myrosinase-catalysed derived products of four thio-functionalised glucosinolates on 10 soil-borne pathogens

2.2 In-vivo assay. The in-vivo fungitoxic activity was assayed toward *Pythium* spp using the biomass of *Iberis amara* L (ISCI 10) and *Rapistrum rugosum* L cv (ISCI 3), selected for their relatively high content of GIB and GCH respectively. These plants, which also contained myrosinase, were ploughed into a naturally infected soil in a calculated amount based on their usual field production in Northern Italy. For the assay, fresh chopped tissue was mixed with soil (clayey soil, pH 8) in pots to obtain GIB 0.20 μmol g^{-1} of soil and GCH 0.26 μmol g^{-1} of soil.[13] Fresh sunflower tissue was used as a control. The extent of test fungus inoculum in treated soil was determined at the beginning and after three weeks using the plate dilution method on selective media.[14] Results were reported as Effectiveness Index [EI(%)] as compared with the control.

3 RESULTS AND DISCUSSIONS

3.1 In-vitro assay. The ThioGL-DPs proved to be strong growth inhibitors of the soil-borne fungi tested. The factorial analysis of EI(%) at 0.2 mM of ThioGL-DPs activity showed that fungal sensitivity varied according to the species (Table 1). The fungitoxic activity of GIB- and GCH-DPs was similar, but significantly higher than that observed with the other two ThioGL-DPs. Finally, the fungus–product interaction was highly significant (Table 1).

Table 2 shows that *Pythium* and *Phytophthora* spp were the most sensitive species, since neither grew on media poisoned with 0.2 mM of GIB- and GCH-DPs, while the growth of *Phytophthora* spp was completely inhibited at doses lower than 0.025 mM. *Sclerotium rolfsii* Sacc, one of the most sensitive fungi, showed a strong reduction of sclerotia production at doses higher than the EC_{50}. The ThioGL-DP activity not only reduced radial growth and number of *S. rolfsii* sclerotia, but also induced a delayed maturation and abnormally shaped sclerotia. In addition, *Rhizoctonia solani* Kühn, one of the main worldwide soil-borne pathogens, showed a high sensitivity to ThioGL-DPs (Tables 1 and 2).

3.2 In-vivo assay. In soil, the treatment with both GIB- and GCH-containing plants reduced the number of *Pythium* propagules with an EI of 88%. GIB- and GCH-DPs in soil reduced *Pythium* spp at a dose very close to that observed *in vitro*. (Tables 1 and 2). This finding indicated that the organic and inorganic components of soil did not interfere with the myrosinase-catalysed hydrolysis of ThioGL or with the inhibition activity of their DPs.

4 CONCLUSIONS

The fungitoxicity, the wide activity spectrum and the physicochemical properties of ThioGL-DPs suggest the potential use of these molecules as efficient alternatives to commercial fumigants for controlling several soil-borne pathogens. These findings suggest some practical applications such as green manuring with plants with a high ThioGL-content. The different sensitivities of soil-borne fungal pathogens suggest an interesting possibility for the control of Oomycetes, *Rhizoctonia* spp and *S rolfsii* in combination with such biological control methods as using antagonistic fungi belonging to Deuteromycetes. In fact, the latter were at least 10 times less sensitive than other pathogenic fungi, as shown in this study. Finally, the use of ThioGL-DPs appears to be a good compromise between the need to use fungitoxic compounds to control soil-borne pathogens and environment protection requirements.

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An Umpolung approach to fluorinated non-ester pyrethroids

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Abstract: The synthesis of novel, fluorinated non-ester pyrethroids by electrophilic fluorination of a stabilised anion with *N*-fluorobenzenesulfonimide is reported.

Keywords: non-ester pyrethroids; electrophilic fluorination

1 INTRODUCTION

The introduction of fluorine into biologically active compounds is of interest as it alters the metabolism, stereoelectronics and lipophilicity of the molecule, yet is isosteric to the proton equivalent. In the case of pyrethroid insecticides, introduction of fluorine also alters the spectrum of activity.[1] However, no information is available on the effect of fluorination at the benzylic position of the central region. The nature of the substituents at this position is known to alter the activity of pyrethroids, especially towards resistant strains of insects.

2 EXPERIEMENTAL

The fluorine anion is a poor nucleophile and often requires special reagents for its introduction, such as diethylaminosulfur trifluoride (DAST), which participates in the reaction by supplying the fluorine atom and creating an excellent leaving group. The generation of this leaving group prevents the use of DAST in the synthesis of non-ester pyrethroids because undesirable alkene or cyclopropane rearrangement[2] and *ipso* reaction of the aromatic groups may all occur under these conditions. In contrast, a cyano group at the benzylic position, known to enhance insecticidal activity in many pyrethroids, can stabilise a negative charge thus allowing fluorine to be introduced regioselectively under *electrophilic* conditions. Of the several reagents available for this transformation we chose *N*-fluorobenzenesulfonimide.

Alkenes **1a**–**f** (Fig 1) and alkanes **3a**,**b**,**f** (Fig 1) are either known compounds or analogues of known compounds and were prepared by literature procedures. Deprotonation of alkenes **1a**–**e** was achieved by lithium bis(trimethylsilyl)amide to produce a delocalised anion with negative charge expected to be stabilised *alpha* to the cyano group. Introduction of the fluorine electrophile yielded only one detectable regioisomer (**2a**–**e**) with concomitant deconjugation to generate an (*E*)-olefin. The fluorination reaction was also performed successfully on compounds

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